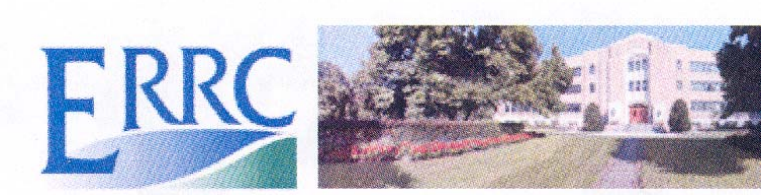

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Title: Method of Applying Sanitizers and Sample Preparation Affects Recovery of Native Microflora and *Salmonella* on Whole Cantaloupe Surfaces

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Research Note

Method of Applying Sanitizers and Sample Preparation Affects Recovery of Native Microflora and *Salmonella* on Whole Cantaloupe Surfaces†

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ABSTRACT

Standardized methods for applying sanitizer treatments to cantaloupes and for recovering surviving native microflora or *Salmonella* on inoculated cantaloupe after sanitizing are lacking. Accordingly, the objectives of this study were to compare four methods for applying sanitizers (dipping, dipping with rotation, dipping with agitation, and dipping with rubbing) using 200 ppm of chlorine or 5% H_2O_2, two recovery methods (homogenization of rind plugs in a stomacher or blender), and five selective recovery media for *Salmonella*. Whole cantaloupes were submerged in a cocktail of five strains of *Salmonella* (each at approximately 2×10^8 CFU/ml) for 10 min and allowed to dry for 1 h inside a biosafety cabinet and stored at 20°C for approximately 23 h before sanitizing. The recovery of *Salmonella* from whole cantaloupe without sanitizing averaged 5.09 log CFU/cm^2 by blending and 4.30 log CFU/cm^2 by homogenization in a stomacher for the five selective agar media. Microbial populations (*Salmonella* or the indigenous aerobic mesophilic bacteria, gram-negative bacteria, lactic acid bacteria, *Pseudomonas* spp., and yeast and mold) were not significantly ($P > 0.05$) reduced by treating with water regardless of the treatment method used. Sanitizing with chlorine or H_2O_2 by dipping, with or without rotation for 2 min, also did not reduce microbial populations. However, populations of all classes of native microflora and *Salmonella* were significantly ($P < 0.05$) reduced by sanitizer treatments (2 min) applied with agitation or by rubbing. In general, sanitizer treatments applied by rubbing resulted in greater log reductions (by up to 1.7 log unit) than for treatments applied with agitation. Populations of native microflora and *Salmonella* recovered from cantaloupe were higher (by up to 1.8 log unit) by blending compared to homogenization in a stomacher. In most instances, selective media used did not differ significantly ($P > 0.05$) for recovery of *Salmonella* after washing treatments.

There are many reports of disease due to consumption of fruits and vegetables that were contaminated at the surface with enteric pathogens *(2)*. Salmonellosis has been steadily increasing as a public health problem in the United States since reporting began in 1943 *(14)*. *Salmonella* is among the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States *(10)*. Five multistate outbreaks of salmonellosis have been associated epidemiologically with cantaloupes. The first in 1990 involved *Salmonella* Chester, which affected 245 individuals (two deaths) in 30 states *(13)*. The second in 1991 involved more than 400 laboratory-confirmed *Salmonella* Poona infections and occurred in 23 states and Canada *(5)*. The most recent multistate outbreaks (occurring in 2000, 2001, and 2002) were due to *Salmonella* Poona *(6)*. Transfer of *Salmonella* from the cantaloupe rind into the flesh by the physical act of cutting the whole cantaloupe or direct contact with contaminated rinds has been reported *(17)*. Therefore, the safety of fresh and fresh-cut produce available in salad-bar operations and supermarkets is a concern *(7, 9)*.

Estimation of microbial populations on a foodstuff is problematic, particularly where the surface of interest is highly morphologically diverse as in the case of cantaloupe due to the extensive netting. Complete recovery of bacteria or other microorganisms from cantaloupe surfaces is problematic due to the surface roughness *(16)*. The surface roughness favors microbial attachment and complicates detachment. A single protocol is, most likely, not suitable for all fruits and vegetables. The development of new methods and validation of new and standard methods to accurately determine populations of a number of pathogenic microorganisms on raw fruits and vegetables are needed *(3)*.

Our objectives were to determine which sanitizing methods (dipping with or without rotation, agitation, or rubbing) were most effective for reducing microbial populations and the effect of sample preparation methods (homogenization in a stomacher or blender) on recovery of *Salmonella* and native microflora from inoculated cantaloupes. Also, the effect of selective plating media on pathogen recovery was evaluated.

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† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and inoculum preparation. Bacterial strains used in this study were *Salmonella* Stanley H0558, *Salmonella* Newport H1275, *Salmonella* Anatum F4317, *Salmonella* Infantis F4319 (all associated with alfalfa sprout–related outbreaks and obtained from Dr. Patricia Griffin, Centers for Disease Control and Prevention), and *Salmonella* Poona RM2350 (associated with the year 2000 cantaloupe-related outbreak and obtained from Dr. Robert Mandrell, Western Regional Research Center, Agricultural Research Service). Bacteria were maintained on brain heart infusion agar (BBL/Difco, Becton Dickinson, Sparks, Md.) slants held at 4°C. Before use, the cultures were subjected to two successive transfers by loop inocula to 5 ml of brain heart infusion broth (BBL/Difco). A final transfer of 0.2 ml was made into 20 ml of brain heart infusion broth with incubation at 36°C for 18 h under static conditions. Bacterial cells were harvested by centrifugation (10,000 × *g*, 10 min) at 4°C, and the cell pellets were washed in salt peptone (0.85% NaCl, 0.05% Bacto-peptone [BBL/Difco]). A cocktail of *Salmonella* containing approximately 2.0×10^8 CFU/ml of each strain was prepared in 3 liters of 0.1% (wt/vol) peptone water.

Inoculation of cantaloupe. Unwaxed, whole cantaloupes (1,631 to 1,743 g, western shippers) purchased from a local produce warehouse were allowed to come to room temperature (approximately 20°C) overnight before being inoculated. Cantaloupes were submerged in 3 liters of bacterial inoculum (approximately 18°C) and agitated by stirring with a glove-covered hand for 10 min. The inoculated cantaloupes were air dried for 1 h in a biosafety cabinet and then stored at 20°C for 24 h before treatments were applied.

Sanitizer treatments. Three treatments were compared: sterile tap water, 200 ppm of chlorine, and 5% hydrogen peroxide. The 200-ppm chlorine solution was prepared by diluting Clorox commercial bleach containing 5.25% NaOCl in sterile deionized water and adjusting the pH to 6.4 ± 0.1 by adding citric acid (Mallinckrodt, Paris, Ky.). Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, Iowa) that has been approved by the U.S. Environmental Protection Agency. A 5% hydrogen peroxide solution was prepared from a 30% stock solution (Fisher Scientific, Suwanee, Ga.) by dilution with sterile tap water.

Four treatment methods (dipped in sanitizer solution, dipped in sanitizer solution with rotation by hand, dipped in sanitizer solution with constant agitation by hand, or dipped in sanitizer solution with continuous rubbing of the cantaloupe surface with glove-covered hand, all 2 min in duration) were compared. Cantaloupes were treated individually in 3 liters of water or freshly prepared sanitizer solution, and treated cantaloupes were then placed inside a biosafety cabinet to dry for 1 h before sample preparation.

Sample preparation and enumeration of microflora. A sterilized stainless steel cork-borer was used to cut randomly through the cantaloupe surface to produce rind plugs of 22 mm in diameter with a surface area (πr^2) of 3.80 cm^2. A total of 152 rind plugs per cantaloupe were obtained. The flesh adhering to the rind plugs was trimmed off using a sterilized stainless steel knife.

Random samples of 70 plugs per cantaloupe weighing approximately 25 g total were either blended (Waring commercial blender, speed level 5, 1 min) along with 75 ml of 0.1% peptone water or placed in a stomacher bag (Dynatech Laboratories, Alexandria, Va.) along with 75 ml of 0.1% peptone water and pummeled for 30 s in a stomacher (model 400, Dynatech Laboratories) at medium speed. Decimal dilutions of the samples were made with 0.1% peptone water, and 0.1 ml was plated in duplicate on various agar media. Two cantaloupes per treatment were sampled in each of three separate experiments. Plate count agar (PCA) with incubation at 30°C for 72 h was used for enumeration of mesophilic aerobic bacteria. PCA with 2 mg/liter of crystal violet added with incubation at 30°C for 72 h was used for gram-negative bacteria. deMan Rogosa Sharpe plus 0.08% sorbic acid with an overlay of the same media in an anaerobic chamber with incubation at 30°C for 48 h was used for lactic acid bacteria. Czapek malt agar with incubation at 30°C for 72 h was used for yeast and mold. *Salmonella* was enumerated on hektoen enteric, mannitol lysine crystal violet brilliant green, xylose lysine desoxycholate, bismuth sulfite, and *Salmonella* Shigella agars with incubation at 35°C for 48 h. All media were obtained from BBL/Difco. For comparison, a pure culture of *Salmonella* Poona RM 2350 was plated on all five selective media and run parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* according to the *FDA Bacteriological Analytical Manual* following conventional biochemical methods *(1)*, as well as serological assays using latex agglutination (Oxoid, Ogdensburg, N.Y.).

TABLE 1. *Effect of sample preparation on recovery of naturally occurring microflora on unwashed whole cantaloupe rind*

Spoilage flora	Log CFU/cm^2[a]	
	Stomacher	Blender
Mesophilc aerobes	5.89 ± 0.17 B	6.92 ± 0.13 A
Gram-negative bacteria	2.89 ± 0.27 B	3.93 ± 0.15 A
Lactic acid bacteria	3.85 ± 0.20 B	4.79 ± 0.10 A
Pseudomonas spp.	1.64 ± 0.13 B	2.52 ± 0.11 A
Yeast and mold	2.51 ± 0.10 B	3.39 ± 0.17 A

[a] Values are mean ± SD of three experiments with duplicate determinations per experiment. Means in each row not followed by the same letter are significantly ($P < 0.05$) different.

Statistical analyses. All experiments were performed in triplicate with duplicate samples analyzed at each sampling time. Data were analyzed with SAS statistical software (SAS Institute, Cary, N.C.) for analysis of variance and the Bonferroni least significant difference method *(11)* to determine if there were significant differences ($P < 0.05$) between mean values of number of cells recovered after each treatment.

RESULTS AND DISCUSSION

Effect of sample preparation and treatment method on recovery of native microflora. The initial level of mesophilic aerobic bacteria recovered from the surface of untreated cantaloupes is shown Table 1. In almost all cases, populations of the five classes of native microflora (aerobic mesophilic bacteria, gram-negative bacteria, lactic acid bacteria, *Pseudomonas* spp., and yeast and mold) recovered from cantaloupe rinds were higher (by up to 1.50 log unit) by blending compared with homogenization in a stomacher regardless of the treatment methods used (Tables 1 through 3). For *Pseudomonas* spp. and yeast and mold, the differences were not always significant, however (Tables 2 and 3). Treatments with water regardless of the method used did not cause a significant ($P > 0.05$) reduction of any class

TABLE 2. *Recovery of naturally occurring microflora on whole cantaloupe rind dipped in 200 ppm of chlorine*

Method of treatment[a]	Log CFU/cm²[b]			
	Stomacher	Log reduction[c]	Blender	Log reduction
Constant agitation				
Mesophilic aerobes	2.49 ± 0.13 B	3.4	3.86 ± 0.13 A	3.0
Gram-negative bacteria	1.60 ± 0.20 B	1.3	2.84 ± 0.14 A	1.0
Lactic acid bacteria	1.95 ± 0.10 B	1.9	2.89 ± 0.16 A	2.0
Pseudomonas spp.	1.02 ± 0.11 B	0.6	1.48 ± 0.10 B	1.0
Yeast and mold	0.68 ± 0.14 B	1.8	0.81 ± 0.16 B	2.5
Constant rubbing				
Mesophilic aerobes	2.09 ± 0.10 B	3.8	3.56 ± 0.16 A	3.4
Gram-negative bacteria	1.30 ± 0.10 B	1.5	2.34 ± 0.12 A	1.6
Lactic acid bacteria	1.45 ± 0.12 B	2.4	2.29 ± 0.13 A	2.5
Pseudomonas spp.	0.86 ± 0.11 B	0.8	0.98 ± 0.10 B	1.5
Yeast and mold	0.38 ± 0.12 B	2.2	0.51 ± 0.16 B	2.8

[a] Treatments applied for 2 min.

[b] Values are mean ± SD of three experiments with duplicate determinations per experiment. Means in each row not followed by the same letter are significantly ($P < 0.05$) different.

[c] Log reductions determined by comparison to populations determined for untreated cantaloupe.

of native microflora on the cantaloupe surfaces (data not shown).

Treatments with 200 ppm of chlorine or 5% H_2O_2 with or without rotation did not cause a significant ($P > 0.05$) reduction of the surface native microflora on cantaloupe (data not shown). Treatments using 200 ppm of Cl_2 or 5% H_2O_2 with constant agitation or rubbing for 2 min caused significant ($P < 0.05$) reductions for all five classes of native microflora (Tables 2 and 3). The log reductions noted were dependent on the method of sample preparation.

Effect of sample preparation and washing method on recovery of *Salmonella*. Populations of *Salmonella* recovered from the surfaces of untreated, inoculated, whole cantaloupe by blending or homogenization in a stomacher averaged 5.09 log CFU/cm² and 4.30 log CFU/cm² for all five selective media used, respectively. In most cases, there were no significant differences ($P > 0.05$) in the populations determined between media (Tables 4 and 5). In almost all instances, *Salmonella* populations recovered from cantaloupe rinds were higher (by up to 1.8 log unit) after homogenization with a blender compared with a stomacher regardless of the treatment method used. Populations of *Salmonella* were not significantly ($P > 0.05$) reduced by treating with water by the treatment methods used.

Treatment with 200 ppm of chlorine or 5% hydrogen peroxide by dipping for 2 min with or without rotation also did not result in significant ($P > 0.05$) reductions of *Salmonella* on the cantaloupe rinds (data not shown). Chlorine treatment with constant agitation and sample homogenization in a stomacher yielded an average recovery of 2.0 to

TABLE 3. *Recovery of naturally occurring microflora on whole cantaloupe rind dipped in 5% hydrogen peroxide*

Method of treatment[a]	Log CFU/cm²[b]			
	Stomacher	Log reduction[c]	Blender	Log reduction
Constant agitation				
Mesophilic aerobes	2.19 ± 0.11 B	3.7	3.59 ± 0.13 A	3.4
Gram-negative bacteria	1.39 ± 0.13 B	1.5	2.34 ± 0.14 A	1.6
Lactic acid bacteria	1.47 ± 0.12 B	2.4	2.63 ± 0.16 A	2.1
Pseudomonas spp.	0.92 ± 0.11 B	0.7	1.18 ± 0.10 B	1.4
Yeast and mold	0.88 ± 0.14 B	1.7	0.91 ± 0.16 B	2.4
Constant rubbing				
Mesophilic aerobes	2.00 ± 0.11 B	3.9	3.24 ± 0.10 A	3.7
Gram-negative bacteria	1.13 ± 0.10 B	1.7	2.07 ± 0.14 A	1.9
Lactic acid bacteria	1.07 ± 0.10 B	2.8	2.40 ± 0.12 A	2.5
Pseudomonas spp.	0.97 ± 0.13 B	0.6	1.00 ± 0.11 B	1.5
Yeast and mold	0.93 ± 0.12 B	1.6	0.86 ± 0.12 B	2.4

[a] Treatments applied for 2 min.

[b] Values are mean ± SD of three experiments with duplicate determinations per experiment. Means in each row not followed by the same letter are significantly ($P < 0.05$) different.

[c] Log reductions determined by comparison to populations determined for untreated cantaloupe.

TABLE 4. *Influence of selective media and method of treatment application on recovery of* Salmonella *from sanitized cantaloupe rind dipped in 200 ppm of chlorine*

Method of treatment[a]	Log CFU/cm²[b] Stomacher	Apparent log reduction[c]	Blender	Apparent log reduction
Constant agitation				
Bismuth sulfite agar	2.10 ± 0.1 a B	2.2	2.90 ± 0.12 a A	2.2
Mannitol lysine crystal violet brilliant green agar	2.00 ± 0.13 a B	2.3	2.68 ± 0.14 a A	2.4
Xylose lysine desoxycholate agar	2.23 ± 0.13 a B	2.0	2.15 ± 0.13 a B	2.9
Hektoen enteric agar	2.14 ± 0.11 a B	2.2	2.83 ± 0.12 a A	2.3
Salmonella Shigella agar	2.10 ± 0.11 a B	2.2	2.76 ± 0.12 a A	2.3
Constant rubbing				
Bismuth sulfite agar	1.13 ± 0.11 b B	3.2	1.69 ± 0.14 b A	3.4
Mannitol lysine crystal violet brilliant green agar	1.19 ± 0.10 b B	3.1	1.56 ± 0.15 b A	3.5
Xylose lysine desoxycholate agar	1.09 ± 0.14 b B	3.2	1.59 ± 0.14 b A	3.5
Hektoen enteric agar	1.10 ± 0.11 b B	3.2	1.69 ± 0.12 b A	3.4
Salmonella Shigella agar	1.00 ± 0.11 b B	3.3	1.62 ± 0.12 b A	3.5

[a] Treatments applied for 2 min 24 h after inoculation.
[b] Values are mean ± SD of three experiments with duplicate determinations per experiment. Means in each row or column not followed by the same letter (lowercase for columns, small caps for rows) are significantly ($P < 0.05$) different.
[c] Apparent log reductions determined by comparison to populations on untreated inoculated rind.

2.3 log CFU/cm² of *Salmonella* on cantaloupe rind independent of the selective medium tested (Table 4). When similar samples were homogenized by blending, the population of *Salmonella* recovered was generally higher (up to 0.8 log). The apparent population reductions ranged from 2.0 to 2.9 log CFU/cm² based on the recoveries using the various selective media. Chlorine treatments by rubbing were more effective than treatment by agitation (apparent

TABLE 5. *Influence of selective media and method of treatment application on recovery of* Salmonella *from sanitized cantaloupe rind dipped in 5% hydrogen peroxide*

Method of treatment[a]	Log CFU/cm²[b] Stomacher	Apparent log reduction[c]	Blender	Apparent log reduction
Constant agitation				
Bismuth sulfite agar	1.80 ± 0.10 a B	2.2	2.75 ± 0.12 a A	2.2
Mannitol lysine crystal violet brilliant green agar	2.00 ± 0.13 a B	2.3	2.78 ± 0.14 a A	2.4
Xylose lysine desoxycholate agar	1.83 ± 0.13 a B	2.0	2.55 ± 0.13 a A	2.9
Hektoen enteric agar	1.14 ± 0.11 b B	2.2	2.96 ± 0.12 a A	2.3
Salmonella Shigella agar	1.10 ± 0.11 b B	2.2	2.96 ± 0.12 a A	2.3
Constant rubbing				
Bismuth sulfite agar	0.83 ± 0.11 b B	3.5	1.18 ± 0.13 b A	3.9
Mannitol lysine crystal violet brilliant green agar	0.79 ± 0.10 b B	3.5	1.18 ± 0.12 b A	3.9
Xylose lysine desoxycholate agar	0.89 ± 0.14 b B	3.4	1.29 ± 0.14 b A	3.8
Hektoen enteric agar	0.80 ± 0.11 b B	3.5	1.20 ± 0.12 b A	3.9
Salmonella Shigella agar	0.80 ± 0.11 b B	3.5	1.20 ± 0.12 b A	3.9

[a] Treatments applied for 2 min 24 h after inoculation.
[b] Values are mean ± SD of three experiments with duplicate determinations per experiment. Means in each row or column not followed by the same letter (lowercase for columns, small caps for rows) are significantly ($P < 0.05$) different.
[c] Apparent log reductions determined by comparison to populations on untreated inoculated rind.

log reductions of 3.1 to 3.5 log CFU/cm^2). Once again, higher bacterial recoveries were obtained by blending of samples and the selective agar media used for plating had no effect (Table 4).

As for the chlorine treatments, sampling of inoculated melon rinds sanitized with hydrogen peroxide by constant agitation or rubbing treatments by blending resulted in significantly ($P < 0.05$) higher recoveries of surviving *Salmonella* than by stomaching (Table 5). Also, as for the chlorine treatments, applying hydrogen peroxide by rubbing the surface of the inoculated melons was significantly more effective than agitation in the sanitizer (Table 5).

The efficacy of treatments on detachment or inactivation of *Salmonella* on cantaloupe surfaces is dependent on the state and location of the organisms on the outer surface and the time of treatment after contamination *(17)*. In this study, the efficacy of treating laboratory-inoculated whole cantaloupes in chlorinated (200 ppm) water or 5% hydrogen peroxide 24 h after inoculation was dependent on the method of applying the sanitizing treatment (Tables 4 and 5). Also, the method of sample preparation (homogenization in a stomacher or blender) affected the total numbers of surviving bacteria enumerated. Log reductions for *Salmonella* on cantaloupe surface were similar; however, more *Salmonella* was recovered from the rind by homogenization in a blender than in a stomacher for the control and sanitized cantaloupe. In general, sanitizer treatments with rubbing resulted in the greatest log reductions for all classes of native microflora and for *Salmonella*. Application of treatments with agitation was less effective than rubbing, and dipping in sanitizers with or without rotation was totally ineffective.

Harris et al. *(8)* recommended the use of a rubbing treatment as a standard method for testing the efficacy of sanitizers on tomatoes and other fruits and vegetables with similar rigid, smooth surfaces. Factors that influence the efficacy of sanitizers for produce include differences in surface morphology, method of applying inoculum, procedures for preparing and applying inoculum, and methods for removal and enumeration of surviving cells *(12)*.

Our studies also indicate that method of sample preparation affects the level of recovery of inoculated populations of *Salmonella* and the native microflora of cantaloupe. Homogenization of cantaloupe rinds with a blender rather than a stomacher led to a greater recovery of native microflora and inoculated *Salmonella*. In contrast, Burnett and Beuchat *(4)* reported similar recoveries, in most cases, of *Salmonella* inoculated onto several types of produce by homogenizing in a blender compared with a stomacher. Cantaloupe was not included in their study. Also, Wu et al. *(18)* reported no difference in recovery of *Escherichia coli* O157:H7 on inoculated alfalfa seed using the two methods of homogenization. Thus, the effect of homogenization method appears dependent on the plant materials tested. The hard, uneven rind of cantaloupe may not be thoroughly homogenized in a stomacher when pummeled for 30 s, and homogenization of rind in a stomacher for 30 s may not lead to the release of tightly attached bacteria in contrast to homogenization with a blender. An alternative treatment affecting recovery of inoculated bacteria may be the method of inoculation (dipping versus spot or spray inoculation) *(3)*.

Previously, we reported significant reduction of *Salmonella* Stanley *(17)* and native microflora on whole cantaloupe surfaces after treatment with chlorine or hydrogen peroxide with rotation for 5 min *(15, 16)* in contrast to the current study. The significant reductions noted in our previous studies could be attributed to the increased length of contact of cantaloupe with the sanitizers compared with the current study (2 min).

In conclusion, based on the methods compared in this study, homogenization with a blender rather than a stomacher led to greater recoveries of both native microflora and inoculated bacterial human pathogens. In addition, application of sanitizer to whole cantaloupe surfaces was more effective when the surface was rubbed.

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